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Aspergillus niger van Tieghem AS TOXIC AND ALLERGENIC MOULD IN DWELLING BUILDINGS AFTER FLOODS

Key words: moulds, housing buildings, flood

Summary

In last years problems of moulds in dwelling buildings becomes very important and vide broached in Europe and Poland territory. It’s not only for moulds in dampness buildings, but also as effect such disasters like floods. 76 species of moulds were detected on walls in dwellings therein from buildings after floods in the Lubuskie Province of which ten are the most common ones. Among those ten moulds, there are six species that, in the order of the most common allergens, cause an inflammatory condition, namely: Penicillium chrysogenum, Aspergillus niger, Cladosporium herbarum, Aspergillus versicolor, Alternaria alternata, Aspergillus flavus. Four of them were selected for biotoxicological tests: three belonging to the class of Aspergillus and one belonging to the class of Cladosporium (Table 1). The method of the bioindicating test applying Dugesia tigrina Girard (Plathelminthes, Turbellaria, Tricladida; Paludicola) was used to determine the toxicity of the selected moulds. All bio-tests gave a positive result. It means that moulds synthesise toxins that increase the danger for the inhabitants of the infected buildings (both toxicological and allergic danger). Toxicological and allergenic danger increase in dwelling buildings after floods on account of much more species of moulds. The species that proved to be the most toxic ones were Aspergillus niger and Aspergillus versicolor, whose severe toxicological values LC 50 were very similar. On the other hand, A. niger is a species of mould that may appear in infected space along with mites (Acarina) increasing the number of allergens in the housing environment. In the performed tests, the Cladosporium herbarum proved to be the least toxic species of mould, what is good news for the inhabitants of the infected buildings, since the fungus in question is very common in housing construction, its frequency value being 5.

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Introduction

Over the last years, the number of people vulnerable to allergies has increased significantly, actually it doubled last year. The highest number of people suffering of allergies is registered in Great Britain. Allergies are most commonly associated with pollen and spores of moulds in the air, and thus special announcements are broadcast in mass media, and for specific regions of Poland there are calendars of pollen discharge and periods of the highest levels of mould’s spores in the air [Piontek 2004a]. Such announcements present most commonly such species of moulds as Alternaria and Cladosporium. A separate and increasing problem is that of allergies produced by moulds and mites (Acarina) in housing construction. It is also important problem in dwelling housing after floods.

The source of allergens of moulds are proteins. They are one of the four different products of moulds that may present a danger for health [Nielsen, Gravesen, Nielsen, Andersen, Thrane, Frisvad 1999]. Proteins cause immediate reactions within a couple of minutes after the infection (allergies type 1) [Flannigan, McCabe, Mcgarry 1991], [Gravesen, Frisvad, Samson 1994] and secretion of histamine without agency of IgE, non-immunological reaction – pseudo allergies [Larsen, Clementsen, Hansen, Maltbæk, Gravesen, Skov, Norn, 1996].

Inhalation of a large number of spores, fragments of mycelia and other particles floating in the air influences health and this is why we should not expect visible mildew on construction partitions not to have negative influence on the inhabitants of the infected buildings. Mildewed construction partitions give off a huge number of spores (counted in thousands). Their number may periodically increase or decrease depending on the weather, the season, heating season, sporing of mildew fungi and other factors.

Spores of moulds that are released into the air and fragments of mycelia may cause allergic diseases (allergies type 1) that include: bronchial asthma (mould asthma), allergic inflammation of alveoli (alveolitis allergica, extrinsic allergic alveolitis, hypersensivity pneumonitis), allergic rhinitis, atopic conjunctivitis, toxic syndrome caused by organic dust, described in the literature as organic dust toxic syndrome (ODTS), chronic fatigue-like syndrome [Krysinska-Traczyk 2001]. Allergens of moulds that condense in badly ventilated rooms are a real danger for atopic allergy sufferers [Bogacka 1997]. It is well-known that spores of Aspergillus, Cladosporium and Penicillium fungi, which may be encountered in damp buildings, may cause asthma and/or rhinitis among atopic allergy sufferers. Atopic diseases (hay fever, atopic asthma) are suffered by more than 10% of people. Atopic allergy is a hereditary propensity to excessively synthesise IgE antibodies as compared with antigens that are common in
the environment (e.g. moulds and mites) [Nielsen, Gravesen, Nielsen, Andersen, Thrane, Frisvad 1999].

The main reason for allergies in people in the housing construction is supposed to be mites (Acarina). In mildewed flats, the risk of allergy increases, since apart from mites, bacteria and nematodes, there are also moulds. A peculiar biological film is formed with subsequent stages. Many species of moulds are the sustenance of mites, while dead mites, its excrements and exuviae provide fungi with organic substances. In the mycological tests performed by Piontek [Piontek 2004a] in the Lubuskie Province such an inter-species interaction was observed in mildewed flats for a long period of time (couple of years) between Acarina and ten species of moulds: Acremonium strictum, Aspergillus niger, Botrytischum piluliferum, Chaetomium elongatum, Epicoccum nigrum, Penicillium aurantiogriseum, P. chrysogenum, Rhizopus stolonifer, Trichothe
cium roseum and Ulocladiu m botrytis [Piontek 2004a].

Most moulds, including allergenic ones, have the property of synthesis of secondary metabolic substances. What is important is that mycotoxins have immunosuppressive effects on the human immunological system what makes internal organs (e.g. kidneys, liver, and central nervous system) more vulnerable and negatively influences allergic reactions.

According to experimented conducted on animals, the respiratory tract leads toxins to tissues in an exceptionally efficient way. Creasia et al. [Creasia, Thurman, Wannemacher, Bunner 1990] demonstrated on tested animals that inhalation of toxins causes a systematic toxic effect more efficiently than their absorption through the mouth or viscerally [Flannigan 2001]. Since it is a large number of spores inhaled with the air are what causes allergies, it is worthwhile to relate the two questions — mycotoxicity and allergic reaction related to the presence of moulds in housing construction — and to continue the described investigation.

**Materials and Methods**

What was used in the mycotoxicological tests were four strains of moulds obtained from mass cultures in a malt extract agar (MEA), isolated from housing structures: Aspergillus niger, Cladosporium herbarum, Aspergillus versicolor, Aspergillus flavus (Table 1).

From the obtained biomes of moulds with substrate, water and methanol extracts were prepared GPM (fungi - laboratory substrate-methanol 80%). Toxicological tests of the mildew fungi were carried out using Dugesia tigrina Girard. Toxicity for tested animals was expressed as acute toxicity 240–h LC 50 (mg dm⁻³, %), [Piontek 2004a, Piontek 2004b].
**Aspergillus niger van Tieghem as toxic and allergenic mould...**

Table 1. Allergenic mildew fungi selected for biotoxicological tests

<table>
<thead>
<tr>
<th>Species*</th>
<th>Size of spores</th>
<th>Selected important mycotoxins [Samson et al.,(12)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>3.5 – 5 μm</td>
<td>malformins A,B,C, naftro-γ-pyrons, ochratoxin A</td>
</tr>
<tr>
<td>Cladosporium herbarum</td>
<td>5.5 – 13 x 4-6 μm</td>
<td>-----------</td>
</tr>
<tr>
<td>Aspergillus versicolor</td>
<td>2 –3.5 μm</td>
<td>sterigmatocistine, 5-methoxy-sterigmatocistine</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>3.6 μm</td>
<td>aflatoxin B1, cyclopiaionic acid, 3-nitropropionic acid, sterigmatocistine</td>
</tr>
</tbody>
</table>

* ordered from the most allergenic ones Schata et al.[Schata, Jorde, Elixmann, Linskens 1989]

All species of moulds mentioned in the table were isolated from the lungs during autopsy [Flannigan 2001]. The spores that are most easily inhales are those that are smaller than 7μm and have a small mass, what facilitates their floating in the air.

**Results**

The results of the tests concerned with toxicity of methanol extracts made of biomes of moulds with substrate (GPM) for *Dugesia tigrina* Girard were presented in the order from the most to the least toxic extract 240-h LC 50 (mg dm⁻³, %), i.e. acute toxicity.

Table 2. Results of toxicity of mildew fungi extracts for *Dugesia tigrina* Girard

<table>
<thead>
<tr>
<th>Species</th>
<th>240-h LC 50 (%)</th>
<th>240-h LC 50 (mg dm⁻³)*</th>
<th>Toxicity class**</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger</td>
<td>0.56</td>
<td>69.5</td>
<td>III (moderately poisonous)</td>
</tr>
<tr>
<td>A. versicolor</td>
<td>0.57</td>
<td>70.9</td>
<td>III (moderately poisonous)</td>
</tr>
<tr>
<td>A. flavus</td>
<td>0.85</td>
<td>106.0</td>
<td>IV (barely poisonous)</td>
</tr>
<tr>
<td>C. herbarum</td>
<td>1.24</td>
<td>155.2</td>
<td>IV (barely poisonous)</td>
</tr>
</tbody>
</table>

* biomass of moulds in the extract

**according to the classification by Liebmann [Liebmann 1962]

All extracts proved to be toxic for tested animals. The most toxic ones proved to be *Aspergillus niger* and *Aspergillus versicolor* (values 240-h LC 50...
were very similar, being respectively 0.56 and 0.57%. The extracts proved to be two times more toxic than *Cladosporium herbarum* (240-h LC 50, 1.24%). In the biotoxicological tests in question, that species of fungi proved to be the least toxic mildew fungus, what confirms the fact that it does not synthesize any mycotoxins (Table 1). Between the most and the least toxic extract, there was a GPM extract from the mould called *Aspergillus flavus*, whose 240-h LC 50 was determined to be 0.85%.

![Fig. 1. LC50 values for the tested allergenic species of moulds](image)

The level of toxicity of moulds extracts was evaluated by means of the Liebmann classification [Liebmann 1962]. Moulds extracts were classified as class III of moderately poisonous compounds (*Aspergillus niger* and *Aspergillus versicolor*) and class IV of barely poisonous substances (*Aspergillus flavus* and *Cladosporium herbarum*). None of the tested extracts was not a highly poisonous substance (toxicity class I and II).

**Discussion**

Four kinds of moulds present in housing structures were taken into account in the tests in question. *Cladosporium herbarum* proved to be the least toxic fungus, what is of particular importance for the inhabitants of infected buildings, since the frequency value for that fungus is high: 5 (38.4%). *Cladosporium sp.* produces a few important allergens and more than 30 others that do not have the same clinical importance [Grajewski i Twaružek 2004]. A couple of
metabolic substances (which have antifungal properties and are inhibitors of growth of plants) were isolated from fungi belonging to the genus *Cladosporium* [Nielsen 2002]. Allergenic properties of *Cladosporium* are much lower that in the case of *Alternaria*, but in housing their condensation rises. Tests that were carried out by Piontek [Piontek 2004a] suggest that *Alternaria alternata* occurs in housing construction with the frequency of 2 (5-10%), and consequently *Cladosporium* is considered to be the main source of allergies.

*Aspergillus* sp. is presented as allergic moulds. The species of the genus *Aspergillus* commonly cause asthma and acute syndromes include oedema, some cases may develop into emphysema and allergic inflammation of alveoli [Twarużek 2005]. This is why three species of that genus were included in the tests. They occur with the frequency 3-rather frequently in dwelling houses (A. *flavus* – frequency 14,0%, A. *versicolor* - frequency 13,4%, A. *niger* - frequency 11,0%), [Piontek, Piontek i Bednar 2004].

All species of *Aspergillus* proved to be toxic in the tests in question, what proves that those moulds present a double risk for the inhabitants of infected houses – both in terms of toxicity as well as in those of allergies. The allergic potential increases when mites (*Acarina*) appears along with moulds. Mycological tests executed by Piontek [Piontek 2004a] suggests that such an interaction between species occurred in mildewed flats for a long period between *Acarina* and 10 species of mildew fungi. *Aspergillus niger* is mentioned among those 10 species.


In the presented work, 4 species of mildew fungi were tested from the above mentioned list of allergenic fungi. Among them, the most allergenic one proved to be *A. niger*. At the same time, this is a species that may be found in construction partitions along with mites (*Acarina*). Biotoxicological tests that were carried out suggest that it belongs to toxicity class III (moderately poisonous). Therefore, it causes a double (toxic and allergic) risk that is increased by the correlation with mites.

Epidemiological research and obtaining appropriate extracts from allergenic mildew fungi is particularly difficult. They are concerned with:

- detection of fungus spores and their identification;
– obtaining properly standardised allergenic extract for the purposes of diagnosis and medical treatment, repeatable concentration of main and secondary allergens;
– determination of clinical criteria that will predictably be correlated with the degree of exposure to the allergen of the fungus [Bogacka 1998].

Isolation, cleaning and standardisation of allergens that are synthesized by moulds are the principal problems that make us ignore the value of erroneous measurements in the tests [Jacob, Ritz, Gehring, Koch, Bischof, Wichmann, Heinrich 2002].

Conclusions

Aspergillus niger is a species that may be found in construction partitions along with mites (Acarina). Biotoxicological tests that were carried out suggest that it belongs to toxicity class III (moderately poisonous). This mould produced a highly nephrotoxic component ochratoxin A, which may pose a significant indoor problem. Therefore, causes a double (toxic and allergenic) risk that is increased by the correlation with mites.

Moulds, and therefore also mycotoxins, are present in the environment. If the concentration of spores and fragments of mycelium in the air is preserved at a lower level, it does not cause any reactions in a healthy organism. However, if the concentration rises, then the human and animal organism may be exposed to many diseases: allergies, mycosis, mycotoxicosis. The minimum concentration of mycotoxins of different mildew fungi species in closed rooms and the time of exposure to their effects that may cause disadvantageous results for health. Without a doubt, elimination of moulds, and therefore of mycotoxins and also allergenic proteins from dwelling houses, especially from buildings after floods may reduce the pathogenic symptoms and sometimes eradicate them [Johanning 2002].

References

15. PIONTEK M., PIONTEK R., BEDNAR K.: Alergogenne grzyby pleśniowe w budownictwie mieszkaniowym [Allergenic moulds in dwelling building]
In: Mikotoksyny i patogenne pleśnie w środowisku, VI Międzyn. Konf. Na-
uk.. Akademia Bydgoska 195-199, Bydgoszcz 2004

16. PIONTEK M.: Grzyby pleśniowe i ocena zagrożenia mikotoksycznego w
budownictwie mieszkaniowym [Moulds and estimation of mycotoxic threat
in dwelling buildings]., Wydawnictwo Uniwersytetu Zielonogórskiego,
174pp, Zielona Góra 2004a

17. PIONTEK M.: Zastosowanie biotestu z Dugesia tigrina Girard do badań
mikotoksykologicznych. Cz.I: toksyczność ostra. [Application of the Dugesia
tigrina Girard bioassay in mycotoxicological investigation. Part I: Acute
toxicity] In: Mikotoksyny i patogenne pleśnie w środowisku. VII Konferenc-
ja naukowa.,149-155,Bydgoszcz 2004b

18. SAMSON R.A., HOEKSTRA E.S., FRISVAD J.C., FILTENBORG O.:
Introduction to food – and airborne fungi. Sixth Ed. Utrecht. Centralbureau
voor Schimmelcultures (CBS) 389 pp. The Netherlands 2000

19. SCHATA M., JORDE W., ELIXMANN J.H., LINSKENS H.F.: Allergies to
molds caused by fungal spores in air conditioning equipment. Environment
International 15: 177-179, 1989

20. TWARUŻEK M.: Wykorzystanie biologicznych testów (MTT, Premi®Test)
w ocenie skażeń pomieszczeń mieszkalnych mikotoksynami grzybów
pleśniowych [Using the biological tests (MTT test, Premi®Test) in the
evaluation of mycotoxic contamination of the dwellings]. PhD thesis, Uni-
ersytet Kazimierza Wielkiego, 119 pp, Bydgoszcz 2005

ASPERGILLUS NIGER VAN TIEGHEM JAKO TOKSYCZNY
I ALERGOGENNY GRZYB PLEŚNIOWY W OBIEKTACH BU-
DOWLANYCH PO POWODZIACH

Słowa kluczowe: grzyby pleśniowe, budownictwo mieszkaniowe, powódź

Streszczenie

W ostatnich latach na obszarze Europy i Polski problem z występowana-
kiem grzybów pleśniowych w budynkach mieszkalnych stał się bardzo
ważny i szeroko omawiany. Nie tylko ze względu na występowanie
grzybów pleśniowych w zawiłoconych budynkach, ale także w budyn-
kach uszkodzonych na skutek takich katastrof jak powódź. Na terenie wo-
jewództwa Lubuskiego oznaczono 76 gatunków grzybów pleśniowych
pochodzących z obiektów budowlanych, w tym także z budynków popo-
wodniowych. Spośród oznaczonych gatunków grzybów pleśniowych, 10 z
nich występuje w budownictwie w największą frekwencją. Wśród tych 10
gatunków sześć z nich powoduje alergie. W kolejnością od najbardziej
alerogennych gatunków są to: Penicillium chrysogenum, Aspergillus
Aspergillus niger van Tieghem as toxic and allergenic mould ...